5. CONCLUSION

It is believed that all of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider and withdraw all presently outstanding rejections. It is believed that a full and complete response has been made to the outstanding Office Action and the present application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (248) 641-1600.

Respectfully submitted,

Dated: March 13, 2008

By: _____
G. Gregory Schivley
Reg. No. 27,382

HARNESS, DICKEY & PIERCE, P.L.C.
P.O. Box 828
Bloomfield Hills, Michigan 48303
(248) 641-1600

enclosure:

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Incidence and Prevention of Bacterial Endophthalmitis With the Use of Viscoelastic Materials and Newquinolone

Koichiro Tanaka[1)*] Shinichiro Kobayakawa[1)] Takuya Oshida[1)]
Akiyoshi Tsuji[2)] and Tetsuo Tochikubo[1)]

[1)] 1st Department of Ophthalmology, School of Medicine, Faculty of Medicine, Toho University
[2)] Department of Infection Control and Prevention, School of Nursing, Faculty of Medicine, Toho University

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Original Article

Incidence and Prevention of Bacterial Endophthalmitis With the Use of Viscoelastic Materials and Newquinolone

Koichiro Tanaka[1)]* Shinichiro Kobayakawa[1)] Takuya Oshida[1)]
Akiyoshi Tsuji[2)] and Tetsuo Tochikubo[1)]

[1)] 1st Department of Ophthalmology, School of Medicine, Faculty of Medicine, Toho University

[2)] Department of Infection Control and Prevention, School of Nursing, Faculty of Medicine, Toho University

ABSTRACT

Purpose: We previously reported a delay in newquinolone penetration with viscoelastic materials *in vitro*. In the present study, we attempted to determine the effect of viscoelastic materials on bacterial endophthalmitis and to evaluate "Antibacterial Visco", a novel mixture of viscoelastic material and levofloxacin.

Methods: 1) We developed an endophthalmitis model utilizing anterior chamber inoculation of methicillin-resistant *Staphylococcus aureus* (MRSA) in rabbit. 2) Three groups were then formed to determine the effect of viscoelastic materials on endophthalmitis. A) Mixed inoculation group: inoculation of a mixture of viscoelastic materials and MRSA; B) Separate inoculation group: inoculation of viscoelastic materials followed by inoculation of MRSA; and C) Bacteria inoculation group: inoculation of MRSA. 3) Finally, the effects of a mixture of viscoelastic materials and levofloxacin on endophthalmitis were evaluated; A) antibacterial visco group, B) an eye drop treatment group, C) a non-treatment group, and D) a bacteria inoculation group.

Results: 1) Endophthalmitis occurred at 10^7 CFU/eye, but not at 10^3 CFU/eye. 2) In the Mixed inoculation group, endophthalmitis occurred at 10^3 CFU/eye. No endophthalmitis occurred in the Separate inoculation group or Bacteria inoculation group. 3) Endophthalmitis was able to be prevented in the antibacterial visco group. However, treatment of endophthalmitis was difficult in the eye drop treatment group.

Conclusion: The viscoelastic material fomented the bacterial endophthalmitis. A mixture of viscoelastic material and levofloxacin is effective on the bacterial endophthalmitis prevention.

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KEYWORDS: endophthalmitis, viscoelastic materials, newquinolone

1) 6-11-1 Omorinishi, Ota, Tokyo 143-8541
2) 4-16-20 Omorinishi, Ota, Tokyo 143-0015
* Corresponding Author: tel:03-3762-4151
e-mail:ktktkt@mail.interq.or.jp

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Postoperative bacterial endophthalmitis is a serious postoperative complication[1,2], although its frequency is only about 0.07% due to recent advances in surgical methods and antibacterial drugs[3-8]. However, when postoperative bacterial endophthalmitis occurs and bacteria reach the vitreous, the prognosis remains extremely poor[3,9-12].

Viscoelastic materials are commonly used in ophthalmic surgery[13,14], and the use of new dispersive viscoelastic materials, in addition to the current cohesive viscoelastic materials, is becoming more widespread[15-17]. To protect the corneal endothelium, dispersive viscoelastic materials are designed to remain in the intraocular space[18-21]. However, the effect of residual viscoelastic material on the incidence of postoperative bacterial endophthalmitis has not been reported. In the previous study, we found that viscoelastic materials delayed antibacterial drug penetration *in vitro*. For the present study, a rabbit bacterial endophthalmitis model was developed, and the effect of viscoelastic materials on bacterial endophthalmitis was examined. In addition, we investigated the effectiveness of a compound we have dubbed "Antibacterial Visco", a mixture of viscoelastic material and levofloxacin in preventing bacterial endophthalmitis.

Methods

1. Development of rabbit bacterial endophthalmitis model

A rabbit bacterial endophthalmitis model was developed and changes in intraocular bacterial count, as well as observational and histopathologic findings were examined.

Laboratory animals: Japanese albino rabbits were maintained in accordance with institutional guidelines and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmologic and Vision Research. The animals were housed in separate cages under a cycle of 12-hour light and 12-hour darkness.

Strain : Methicillin-resistant *Staphylococcus aureus* MK99-3 (MRSA MK99-3) obtained from a patient with ocular infection was used.

Anesthesia : Ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (20 mg/ml) were used. The ratio was 7 : 1, respectively. Intraperitoneal injection of 4 ml was given as general anesthesia.

Bacterial liquid inoculation : General anesthesia was administered to 20 Japanese albino rabbits. Paracentesis of 0.1ml of the aqueous humor was then performed. After that, 0.1 ml of bacterial liquid was injected into the anterior chambers of the rabbit eyes. The inoculation bacteria count was adjusted to either approximately 10^3 CFU/eye (N=10) or 10^7 CFU/eye (N=10).

Eyes were observed for corneal opacity, ciliary injection, hypopyon and discharge. Eyes with at least three of these conditions were classified as endophthalmitic. The rabbits were euthanized by injection into the cardiac sac of 4 ml of thiopental sodium (25 mg/ml) either 6, 12, 24, 48 or 72 hours after inoculation, and ophthalmectomy was performed. Cultures of the aqueous humor (0.05 ml/eye) and vitreous humor were prepared. Histopathologic specimens were then taken from the removed eyeballs. After fixing the eyeballs in 10% formalin immediately after removal, Giemsa staining and Hematoxylin-Eosin staining were performed.

2. Effects of viscoelastic material on bacterial endophthalmitis

After the rabbit bacterial endophthalmitis model was developed, viscoelastic material was added. To examine the effect of viscoelastic materials, the timing of viscoelastic and bacterial liquid inoculation was varied.

The details of the laboratory animals, the bacterial strain and the anesthesia are given in the previous section.

Viscoelastic materials: Healon® (Pfizer, USA)-a hyaluronate sodium solution, and Viscoat® (ALCON, USA)-a sodium hyaluronate and chondroitin sulfate sodium solution-were used.

Bacterial liquid inoculation : General anesthesia was administered to 88 Japanese albino rabbits. Then, the anterior chambers of the rabbit eyes were inoculated with viscoelastic material and/or bacterial liquid. There were three inoculation groups.

A) Mixed inoculation group : The ratio of viscoelastic material to bacterial liquid was 9 : 1. Paracentesis of 0.1 ml of the aqueous humor was performed. The anterior chamber was inoculated with 0.1 ml of the mixed liquid (N=16 ; 8 Healon cases and 8 Viscoat cases). The bacteria count was adjusted

to approximately 100 CFU/eye.

B) Separate inoculation group : 0.1 ml of the aqueous humor was removed by paracentesis. Then, 0.09 ml of viscoelastic material was injected into the anterior chamber, followed by a separate 0.01 ml injection of bacterial liquid (N=64; 32 Healon cases and 32 Viscoat cases). Inoculation of bacterial liquid was done immediately, 6, 12, or 24 hrs after inoculation of viscoelastic materials. The bacteria count was adjusted to approximately 100 CFU/eye.

C) Bacteria inoculation group : 0.1 ml of the aqueous humor was removed by paracentesis. Then, 0.1 ml of bacterial liquid was injected into the anterior chamber (N=8). The bacteria count was adjusted to approximately 100 CFU/eye.

Eyes were observed for corneal opacity, ciliary injection, hypopyon and discharge. Eyes with at least three of these conditions were classified as endophthalmitic. Cultures of aqueous humor (0.05 ml/eye) were made either 24 or 48 hrs after initial inoculation. Rabbits were euthanized 48 hrs after inoculation, and ophthalmectomy was performed. Cultures of the aqueous humor (0.05 ml/eye) and vitreous humor were made. Histopathologic specimens were taken from the removed eyeballs. After fixing the eyeballs in 10% formalin immediately after removal, Giemsa and Hematoxylin-Eosin stainings were performed.

3. Prevention of bacterial endophthalmitis with viscoelastic materials and newquinolone

A rabbit bacterial endophthalmitis model was developed. An antibacterial drug was administered to these rabbits by various methods, and the effectiveness was examined.

Details of the laboratory animals, viscoelastic materials and anesthesia used are given in the previous section.

Antibacterial drug: 0.5% Levofloxacin (LVFX, Cravid®, Santen Pharmaceutical, JAPAN) was used. The highest concentration in aqueous humor (AQCmax) of 0.5% LVFX was 2.17 μg/ml[22].

Strain : *Staphylococcus aureus* Smith was used, because it can be treated with antibacterial drugs and is capable of causing endophthalmitis. The minimum inhibitory concentration of LVFX to *Staphylococcus aureus* Smith was 0.25 μg/ml.

Bacterial liquid inoculation : General anesthesia was given to 52 Japanese albino rabbits. Then, bacterial liquid was injected into the anterior chambers of the rabbit eyes. There were 4 inoculation groups.

A) Antibacterial Visco group (N=20, 10 Healon cases and 10 Viscoat cases): the ratio of viscoelastic material to bacterial liquid to LVFX was 9 ml : 0.5 ml : 0.5 ml. The materials were mixed immediately before inoculation. Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye. The final concentration of LVFX in the mixture was adjusted to 2 μg/ml.

B) Eye Drop Treatment group (N=20, 10 Healon cases and 10 Viscoat cases): the ratio of viscoelastic material to bacterial liquid was 9 ml : 1 ml. The materials were mixed immediately before the inoculation. Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye. 0.5% LVFX eye drop treatment and injection of 50 μl of LVFX into the cul-de-sacs of rabbit eyes 4 times/day had been performed on the day before the inoculation. Eye drop treatment continued until the final day of the study.

C) Non-Treatment group (N=8, 4 Healon cases and 4 Viscoat cases): the ratio of viscoelastic material to bacterial liquid was 9 : 1. The materials were mixed immediately before the inoculation. Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye.

D) Bacteria inoculation group : inoculation of bacterial liquid only (N=4). Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye.

Corneal opacity, ciliary injection, hypopyon and discharge were assessed. Eyes with three or more of these conditions were classified as endophthalmitic. The aqueous humor (0.05 ml/eye) was cultured 24 or 48 hours after inoculation, after general anesthesia. The rabbit was euthanized 48 hours after

inoculation and ophthalmectomy was performed. After general anesthesia, 4 ml of thiopental sodium (25 mg/ml) was injected into the cardiac sac as euthanasia. A culture of the vitreous was then made. The specimens were scanned under scanning electron microscope (Japan Electron Optics Laboratory JSM-5410) at 15 kV.

Bonferroni's method was used for statistical analyses of the difference detection of the population rate of a multi crowd. The significance level was set to 0.01.

Results

1. Development of rabbit bacterial endophthalmitis model

The endophthalmitis rate is shown in Table 1. In the 10^7 CFU/eye inoculation group, endophthalmitis rate at 6 hours after inoculation was calculated and continued thereafter for 72 hours after inoculation. In the 10^3 CFU/eye inoculation group, there were no cases of endophthalmitis during the observation period. The vitreous cultures were positive in all cases of endophthalmitis.

The positive aqueous humor culture rate is shown in Table 2. Aqueous humor cultures in the 10^3 CFU/eye inoculation group were negative after 6 hours and remained negative thereafter. Aqueous humor cultures in the 10^7 CFU/eye inoculation group were positive until 24 hours after inoculation. Thereafter, all aqueous humor cultures were negative, regardless of the severity of endophthalmitis signs. Endophthalmitis findings were examined in histopathologic specimens according to inoculation bacteria count. Edema of the cornea, vasodilation of iris stroma vessel, trabeculum abscess, hypopyon, and bacterial vitreous invasion were observed. Bacterial colonization of the iris was observed in a specimen with a negative aqueous humor culture in the 10^7 CFU/eye inoculation group (Photo 1).

2. Effects of viscoelastic materials on bacterial endophthalmitis

The endophthalmitis rate is shown in Table 3. In the Mixed inoculation group at 100 CFU/eye, the endophthalmitis rate was 8 of 8 eyes and 7 of 8 eyes with Healon and Viscoat, respectively. No endophthalmitis occurred at 100 CFU/eye in the Separate inoculation group or the Bacteria inoculation group. A significant difference in endophthalmitis rate was observed between the Mixed inoculation group and the Separate inoculation group ($p<0.01$, Bonferroni's method). No significant difference in the bacterial endophthalmitis rate was observed between Healon

Table 1 Endophthalmitis rate after inoculation

	10^3 CFU/eye					10^7 CFU/eye				
Observation time after inoculation (Hours)	6	12	24	48	72	6	12	24	48	72
Total eyes	10	8	6	4	2	10	8	6	4	2
Endophthalmitis eyes	0	0	0	0	0	10	8	6	4	2
Crisis (%)	0	0	0	0	0	100	100	100	100	100

Endophthalmitis was present in the 10^7 CFU/eye inoculation group 6 hours after inoculation, and continued thereafter. Endophthalmitis did not develop in the 10^3 CFU/eye inoculation group. All vitreous cultures were positive in cases of endophthalmitis.

Table 2 Rate of positive aqueous humor cultures

	10^3 CFU/eye					10^7 CFU/eye				
Culture time after inoculation (Hours)	6	12	24	48	72	6	12	24	48	72
Total eyes	2	2	2	2	2	2	2	2	2	2
Culture positive eyes	0	0	0	0	0	2	2	2	0	0
Mean CFU (CFU/0.05ml)						1000	500	380		
Culture positive rate (%)	0	0	0	0	0	100	100	100	0	0

All aqueous humor cultures of the 10^3 CFU/eye inoculation group were negative. Aqueous humor culture of the 10^7CFU/eye inoculation group were positive until 24 hours. After that, all aqueous humor cultures were negative, regardless of the severity of endophthalmitis.

Table 3 Endophthalmitis rate after inoculation by groups

	Mixed		Separate								Bacteria
			HL				VIS				
	HL	VIS	0	6	12	24	0	6	12	24	
Total eyes	8	8	8	8	8	8	8	8	8	8	8
Endophthalmitis eyes	8	7	0	0	0	0	0	0	0	0	0
Crisis rate (%)	100	87.5	0	0	0	0	0	0	0	0	0

HL : Healon, VIS : Viscoat
There was a significant difference in endophthalmitis rate between the Mixed inoculation group and the Separate inoculation group ($p<0.01$). There was no significant difference in bacterial endophthalmitis rate between the Healon and Viscoat groups ($p>0.05$). All vitreous cultures of endophthalmitis cases were positive.

Table 4 Positive aqueous humor culture rate by groups

	Mixed				Separate				Bacteria	
	HL		VIS		HL		VIS			
Culture time after inoculation	24	48	24	48	24	48	24	48	24	48
Total eyes	8	8	8	8	32	32	32	32	8	8
Culture positive eyes	8	0	8	0	0	0	0	0	0	0
Mean CFU (CFU/0.05ml)	1245		918							
Crisis rate (%)	100	0	100	0	0	0	0	0	0	0

HL : Healon, VIS : Viscoat
Remarkable bacterial proliferation was observed at 24 hours after inoculation in the Mixed inoculation group. However, aqueous humor cultures were negative at 48 hours. Aqueous humor cultures were negative in the Separate inoculation group and the Bacteria inoculation group.

and Viscoat ($p>0.05$, Bonferroni's method). All vitreous cultures were positive in cases of endophthalmitis.

The aqueous humor culture positive rate is shown in Table 4. Remarkable bacterial proliferation was observed at 24 hours after inoculation in the Mixed inoculation group. However, aqueous humor cultures were negative at 48 hours. Aqueous humor cultures were negative in the Separate inoculation group and the Bacteria inoculation group.

Histopathologic findings of endophthalmitis were noted in the Mixed inoculation group, but not in the Separate inoculation group or the Bacteria inoculation group. After inoculation, residual viscoelastic material was observed. Bacterial proliferation in residual viscoelastic material was observed in the Mixed inoculation group (Photo 2). Limitation of polynuclear leukocyte movement in the viscoelastic material layer was noted in the Mixed inoculation group (Photo 3), i.e., leukocytes did not reach bacteria covered with the viscoelastic material. Bacterial colonization of the ciliary processes was observed in a specimen from a negative aqueous humor culture in the Mixed inoculation group (Photo 4).

3. Prevention of bacterial endophthalmitis with viscoelastic materials and newquinolone

The endophthalmitis rate at 10^4 CFU / eye is shown in Table 5. In the Antibacterial Visco group, 1 of 10 eyes treated with Healon and 2 of 10 eyes treated with Viscoat showed signs of endophthalmitis. In the Eye Drop Treatment group, 9 of 10 eyes treated with Healon and 10 of 10 eyes treated with Viscoat showed signs of endophthalmitis. All vitreous cultures were positive in cases of endophthalmitis.

A significant difference in the endophthalmitis rate was also observed with Healon between the Antibacterial Visco group and the Eye Drop Treatment group ($p<0.01$, Bonferroni's method). There was also a significant difference in endophthalmitis rate with Viscoat between the Antibacterial Visco group and the Eye Drop Treatment group ($p<0.01$, Bonferroni's method). No significant difference was

Table 5 Endophthalmitis rate after inoculation

	Antibacterial Visco		Eye Drop Treatment		Non Treatment		Bacteria
	HL	VIS	HL	VIS	HL	VIS	
Total eyes	10	10	10	10	4	4	4
Endophthalmitis eyes	1	2	9	10	4	4	0
Crisis rate (%)	10	20	90	100	100	100	0

HL : Healon, VIS : Viscoat
There was a significant difference in endophthalmitis rate with Healon between Antibacterial Visco group and Eye Drop Treatment group ($p<0.01$). There was also a significant difference in endophthalmitis rate with Viscoat between Antibacterial Visco group and Eye Drop Treatment group ($p<0.01$). There was no significant difference in endophthalmitis rate between Healon and Viscoat in any group ($p>0.05$). In Non-Treatment group, all eyes suffered endophthalmitis. There was no significant difference in endophthalmitis rate between Non-Treatment group and Eye Drop Treatment group. In Bacteria inoculation group, no eyes contracted endophthalmitis.

observed in endophthalmitis rate between Healon and Viscoat in any group ($p>0.05$, Bonferroni's method). In the Non-Treatment group, all eyes suffered endophthalmitis. No significant difference was observed in endophthalmitis rate between the Non-Treatment group and the Eye Drop Treatment group. In the Bacteria inoculation group, no eyes contracted endophthalmitis.

Aqueous humor cultures taken 24 and 48 hours after inoculation were negative, regardless of endophthalmitic status. Limited migration of polynuclear leucocytes through the viscoelastic material layer was observed (Photo 5). In the Antibacterial Visco group, limited migration of polynuclear leucocytes did not result in endophthalmitis.

Discussion

In the present study using a rabbit bacterial endophthalmitis model, bacterial endophthalmitis occurred at a concentration of 10^7 CFU/eye, but not at 10^3 CFU/eye, a finding which suggests that it is necessary to consider the invading bacterial count after intraocular surgery. Although disinfection and antibacterial drug use are painstakingly utilized to prevent postoperative bacterial endophthalmitis[23-27], many surgeons have reported bacteria spread to the aqueous humor during cataract surgery even when all reasonable preventive measures had been taken[27-36]. Fortunately, invading bacterial counts tend to be very low in recent intraocular surgery[24,30]. Moreover, when bacteria invade, the anterior chamber has stronger resistance than the vitreous. In animal experiments, bacterial endophthalmitis develops at a concentration as low as 10 CFU/eye in vitreous inoculation[37]. A higher number of bacteria are necessary in anterior chamber inoculations: 10,000 or more[38-40]. The results of the present study do not contradict these findings. Our findings suggest that the anterior chamber is an inhospitable location for bacterial proliferation.

However, the reason why the anterior chamber is so resistant to bacterial proliferation is unknown. It has been suggested that clearance activity in the aqueous humor protects against bacterial invasion. Bacteria in the anterior chamber are excluded from the trabeculum by this clearance. Moreover, phagocytes in the trabecula show remarkable phagocytic activity in the eye. A strong inflammatory reaction including ciliary injection is observed in this area in early stage endophthalmitis. Moreover, migration of phagocytes, such as neutrophilic granulocytes, from the vessel of the iris has been noted. Needless to say, antibacterial drug action at surgery might also be important. In the present study, aqueous humor cultures remained negative despite inoculation of bacteria to the anterior chamber and the presence of severe endophthalmitis. Furthermore, bacterial colonization of the iris was observed in a negative aqueous humor culture group. Bacteria in the anterior chamber are not easily detected in an investigation of the aqueous humor. There are many reports suggesting that, in cases of postoperative bacterial endophthalmitis, vitreous cultures are superior to aqueous humor cultures. The results of the present study confirm these reports[5,41]. Our findings

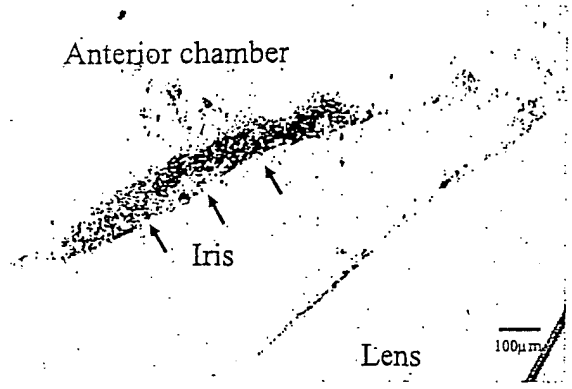
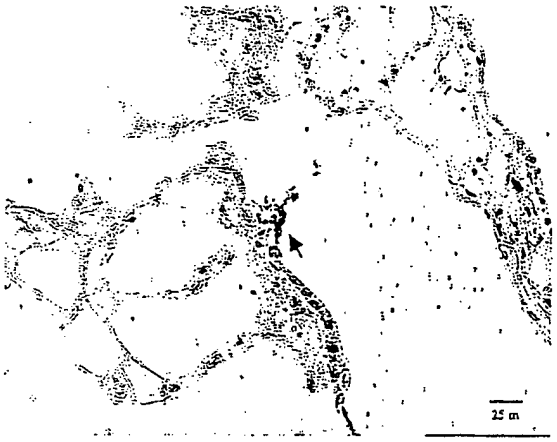


Photo 1



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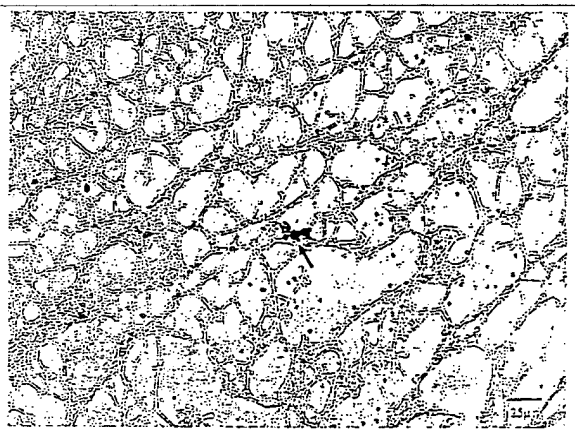

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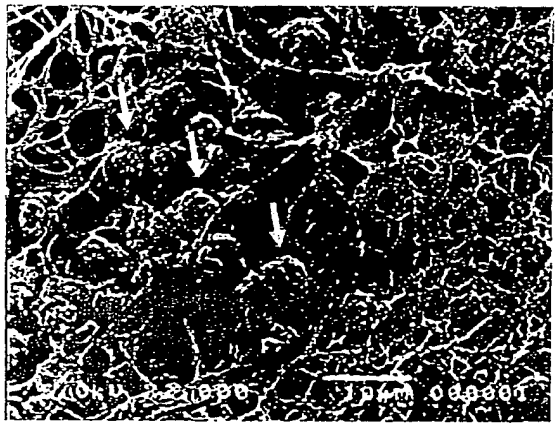
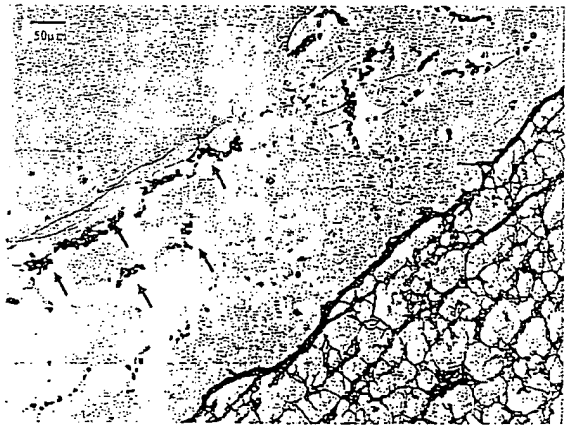


Photo 5



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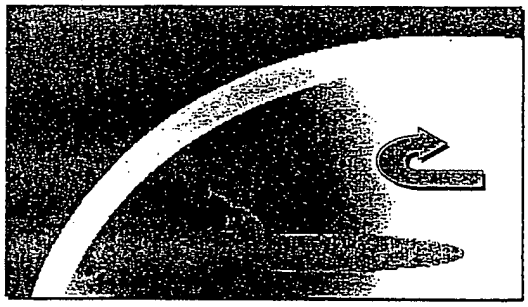

Fig. 1

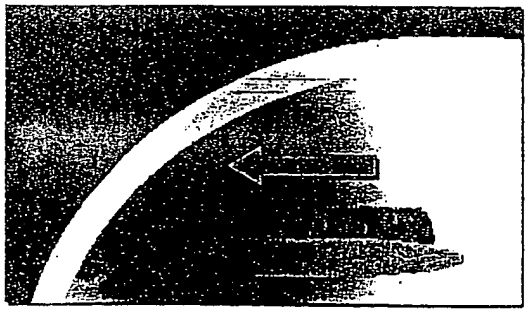

Fig. 2

suggest that through clearance and phagocytosis, the aqueous humor is capable of excluding even large numbers of bacteria. Because of clearance and phagocytosis, the bacterial count decreased with time and negative cultures were obtained after 48 hours in eyes that had been endophthalmitis.

Postoperative bacterial endophthalmitis includes anterior and posterior postoperative bacterial endophthalmitis. Anterior endophthalmitis remains in the anterior chamber, and posterior endophthalmitis reaches the vitreous. Posterior endophthalmitis is refractory, even if emergency vitreous surgery is performed, and visual acuity outcomes are poor. The entry point for bacteria in anterior intraocular surgery such as cataract surgery is the anterior chamber[28]. In modern cataract surgery, the possibility of bacteria immediately reaching the vitreous is very low when rupture of the posterior capsule does not occur[3, 5, 6, 38, 39, 42]. In addition, the number of bacteria invading the anterior chamber is low[30]. However, postoperative bacterial endophthalmitic crises can still occur. The mystery is why a small number of bacteria is capable of evading the robust defenses of the anterior chamber to cause bacterial endophthalmitis.

In the present study of the effects of viscoelastic materials on bacterial endophthalmitis, endophthalmitis occurred at a concentration of 100 CFU/eye in the Mixed inoculation group. Endophthalmitis in the anterior chamber inoculations does not usually develop at this concentration, which suggests that the presence of viscoelastic materials contributes to the incidence of endophthalmitis. However, because endophthalmitis did not develop in the Separate inoculation group in the present study, it appears that viscoelastic materials alone do not make endophthalmitis. There is one report of endophthalmitis due to contaminated viscoelastic material[43]. Residual viscoelastic material in intraocular surgery caused angle occlusion and increased intraocular pressure[44, 45]. Moreover, residual viscoelastic material limits aqueous humor clearance and cause pooling of bacteria by angle occlusion (Fig. 1). In addition, it might shield bacteria from aqueous humor clearance and phagocytosis (Fig. 2). Angle occlusion due to viscoelastic material was present in both the Mixed inoculation group and the Separate inoculation group. However, the protection of bacteria by viscoelastic material in the Mixed inoculation group was more obvious than in the Separate inoculation group; endophthalmitis was indeed observed in the Mixed inoculation group in the present study. Regarding the reason for the occurrence of endophthalmitis with viscoelastic material, the protection of bacteria, rather than angle occlusion, appears more likely. Bacterial proliferation occurs under the viscoelastic material layers.

There was no significant difference in the endophthalmitis rate between Healon and Viscoat in this study. Viscoat, a dispersive viscoelastic material,

Photo 1 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 72 hours after inoculation. (Giemsa stain, ×100)
Bacterial colonization of the iris (arrow) in a specimen from a negative aqueous humor culture from the 10^7 CFU/eye inoculation group.

Photo 2 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 48 hours after inoculation with Viscoat. (Giemsa stain, ×400)
Bacterial proliferation (arrow) can be seen in residual viscoelastic material.

Photo 3 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 48 hours after inoculation with Viscoat. (Hematoxylin-Eosin stain, ×200)
Interruption of polynuclear leukocyte migration (arrow) by viscoelastic material layer occured in the Mixed inoculation group.

Photo 4 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 48 hours after inoculation with Healon. (Giemsa stain, ×400)
Bacterial colonization (arrow) on ciliary processes can be seen in a negative aqueous humor culture from the Mixed inoculation group.

Photo 5 Electron microscopic image of a rabbit eye infected with *Staphylococcus aureus* Smith, 48 hours after the inoculation (×2000).
This image of the iris surface was taken from the corneal side. Retarded migration of polynuclear leucocytes (arrow) through the viscoelastic material layer (net shape) can be seen.

Fig. 1 Residual viscoelastic material can cause pooling of bacteria by angle occlusion

Fig. 2 Residual viscoelastic material might protect bacteria by shielding them from the clearance of aqueous humor flow and phagocytosis.

more readily remains in the intraocular space[16-18], because of its rheological characteristics. Thus it is possible that Viscoat use may result in some degree of endophthalmitis. However, the aspiration procedure usually performed during surgery was not done in the present study. Thus, residue of both viscoelastic materials remained in the anterior chamber in the present study, which may explain the lack of a significant difference between Healon and Viscoat.

In this study, a realistic bacterial count of 100 CFU/eye was inoculated. Other studies of endophthalmitis used anterior chamber inoculations with an unrealistic bacterial count of 10^5 CFU/eye[38-40]. In a recent report, the bacterial count in an aqueous humor culture immediately after cataract surgery corresponded to the bacterial count in the present study[24, 30], suggesting that the possibility of endophthalmitis with a bacterial count of 100 CFU/eye is realistic. In light of these findings, the necessity of viscoelastic material removal becomes clear. However, because complete removal of residual viscoelastic material is impossible, precaution against endophthalmitis appears necessary.

In the present study of prevention of bacterial endophthalmitis with viscoelastic materials in combination with newquinolones, LVFX eye drop treatment was ineffective in preventing endophthalmitis in the presence of viscoelastic material. Although *Staphylococcus aureus* Smith is an LVFX-sensitive strain, LVFX penetration might have been delayed by the presence of viscoelastic material. Indeed, a delay in antibacterial drug penetration by viscoelastic material was reported in the previous article. Because of this delay, the antibacterial drug was washed out by aqueous flow before LVFX penetrated the viscoelastic material. No difference between Healon and Viscoat was observed in the present study because the anterior chamber washing during the actual cataract surgery was not performed in the present study.

Our findings clearly show that Antibacterial Visco, a mixture of viscoelastic material and antibacterial drug, prevented endophthalmitis. In the previous research, we showed that antibacterial drug penetration is not decreased by admixture with viscoelastic material and that viscoelastic material combined with LVFX at AQCmax concentration can prevent bacterial endophthalmitis.

These studies have shown that a small number of bacteria can cause bacterial endophthalmitis in the presence of viscoelastic material, and that the use of viscoelastic material makes antibacterial eye drop treatment ineffective. However, the preventive effect of Antibacterial Visco was demonstrated. Postoperative bacterial endophthalmitis is a very serious complication[1, 2], and a number of possible risk factors have been proposed, including posterior capsular rupture, diabetes mellitus, contamination of drugs or surgical instruments, or insufficient disinfection[19, 46-49]. We investigated the presence of residual viscoelastic material as a risk factor. The followings are tentative explanation for the acceleration of endophthalmitis by viscoelastic materials:

1) Sheltering of bacteria from the clearance effect of aqueous humor by viscoelastic material.

2) Delay in antibacterial drug penetration by viscoelastic material.

These are the negative consequences of viscoelastic material use. However, we believe that such materials can instead be used to prevent endophthalmitis. Antibacterial Visco is an attempt to facilitate antibacterial drug delivery in cases where viscoelastic materials are necessary. Indeed, other applications have already been investigated: anesthetic viscoelastic materials have been reported[50-52], as have viscoelastic materials with gentamicin for bacterial endophthalmitis treatment[53, 54].

The use of viscoelastic material remains indispensable to modern intraocular surgery. In particular, viscoelastic materials are important in cataract surgeries, which represent the majority of intraocular surgeries[13, 14]. Further development of viscoelastic materials is required. One such development was the addition of endothelium protection to the essential space maintenance ability of viscoelastic material [18-21]. The intraocular is not sterile during surgery and the viscoelastic material has not been completely removed at the end of operation. Bacterial endophthalmitis can easily occur in the presence of residual viscoelastic material. In addition, endophthalmitis in the presence of viscoelastic material is refractory to eye drop treatment. In conclusion, our findings show that mixture of antibacterial drug and viscoelastic material can prevent endophthalmitis.

In addition, we believe that development of Antibacterial Visco would be a further improvement. We have already developed a "double protection" viscoelastic material by adding antibacterial action to a viscoelastic which is also capable of protecting the corneal endothelium. We hope that this new viscoelastic material can improve the safety of future intraocular surgical procedures.

References

1) Endophthalmitis Vitrectomy Study Group: Results of the Endophthalmitis Vitrectomy Study. A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis. *Arch Ophthalmol* 113 : 1479-1496, 1995
2) Speaker MG, Menikoff JA: Postoperative endophthalmitis: Pathogenesis, prophylaxis, and management [review]. *Int Ophthalmol Clin* 33 : 51-70, 1993
3) Norregaard JC, Thoning H, Bernth-Petersen P, Andersen TF, Javitt JC, Anderson GF: Risk of endophthalmitis after cataract extraction: Results from the International Cataract Surgery Outcomes study. *Br J Ophthalmol* 81 : 102-106, 1997
4) Kattan HM, Flynn HW Jr, Pflugfelder SC, Robertson C, Forster RK: Nosocomial endophthalmitis survey: Current incidence of infection after intraocular surgery. *Ophthalmology* 98 : 227-238, 1991
5) Aaberg TM Jr, Flynn HW Jr, Scheffman J Jr, Newton J: Nosocomial acute-onset postoperative endophthalmitis survey. A 10-year review of incidence and outcomes. *Ophthalmology* 105 : 1004-1010, 1998
6) Menikoff JA, Speaker MG, Marmor M, Raskin EM: A case-control study of risk factors for postoperative endophthalmitis. *Ophthalmology* 98 : 1761-1768, 1991
7) Allen HF, Mangiaracine AB: Bacterial endophthalmitis after cataract extraction: A study of 22 infections in 20,000 operations. *Arch Ophthalmol* 72 : 454-462, 1964
8) Allen HF, Mangiaracine AB: Bacterial endophthalmitis after cataract extraction II. Incidence in 36,000 consecutive operations with special reference to preoperative topical antibiotics. *Trans Am Acad Ophthalmol Otolaryngol* 77 : OP581-588, 1973
9) Baum JL: Antibiotic administration in the treatment of bacterial endophthalmitis. I. Periocular injection. *Surv Ophthalmol* 21 : 332-346, 1977
10) Forster RK, Abbott RL, Gelender H: Management of infectious endophthalmitis. *Ophthalmology* 87 : 313-319, 1980
11) Ormerod LD, Ho DD, Becker LE, Cruise RJ, Grohar HI, Paton BG, Frederick AR Jr, Topping TM, Weiter JJ, Buzney SM, et al.: Endophthalmitis caused by the coagulase-negative staphylococci. 1. Disease spectrum and outcome. *Ophthalmology* 100 : 715-723, 1993
12) Somani S, Grinbaum A, Slomovic AR: Postoperative endophthalmitis: Incidence, predisposing surgery, clinical course and outcome. *Can J Ophthalmol* 32 : 303-310, 1997
13) Arshinoff SA: Dispersive and cohesive viscoelastic materials in phacoemulsification. *Ophthalmic Pract* 13 : 98-104, 1995
14) Miller D, Stegmann R: Use of Na-hyaluronate in anterior segment surgery. *Am Intraocular Implant Soc J* 6 : 342-343, 1980
15) Glasser DB, Katz HR, Boyd JE, Langdon JD, Shobe SL, Peiffer RI: Protective effects of viscous solution in phacoemulsification and traumatic lens implantation. *Arch Ophthalmol* 107 : 1047-1051, 1989
16) McDermott ML, Hazlett LD, Barrett RP, Lambert RJ: Viscoelastic adherence to corneal endothelium following phacoemulsification. *J Cataract Refract Surg* 24 : 678-683, 1998
17) Poyer JF, Chan KY, Arshinoff SA: Quantitative method to determine the cohesion of viscoelastic agents by dynamic aspiration. *J Cataract Refract Surg* 24 : 1130-1135, 1998
18) Craig MT, Olson RJ, Mamalis N, Olson RJ: Air bubble endothelial damage during phacoemulsification in human eye bank eyes: The protective effects of Healon and Viscoat. *J Cataract Refract Surg* 16 : 597-602, 1990
19) Glasser DB, Osborn DC, Nordeen JF, Min Y-I: Endothelial protection and viscoelastic retention during phacoemulsification and intraocular lens implantation. *Arch Ophthalmol* 109 : 1438-1440, 1991
20) Poyer JF, Chan KY, Arshinoff SA: New method to measure the retention of viscoelastic agents on a rabbit corneal endothelial cell line after irrigation and aspiration. *J Cataract Refract Surg* 24 : 84-90, 1998
21) Ravalico G, Tognetto D, Baccara F, Lovisato A: Corneal endothelial protection by different viscoelastics during phacoemulsification. *J Cataract Refract Surg* 23 : 433-439, 1997
22) Fukuda M, Sasaki K: Antibiotic ophthalmic solutions evaluated by pharmacokinetic parameters of maximum concentration in the aqueous. *Nippon Ganka Gakkai Zasshi* 106 : 195-200, 2002 (J)
23) Assia EI, Jubran RZ, Solberg Y, Keller N: The role of intraocular lenses in anterior chamber contamination during cataract surgery. *Graefes Arch Clin Exp Ophthalmol* 236 : 721-724, 1998
24) Egger SF, Huber-Spitzy V, Scholoda C, Schneider B, Grabner G: Bacterial contamination during extracapsular cataract extraction. *Ophthalmologica* 208 : 77-81, 1994
25) Starr MB: Prophylactic antibiotics for ophthalmic surgery. *Surv Ophthalmol* 27 : 353-373, 1983
26) Speaker MG, Menikoff JA: Prophylaxis of endophthalmitis with topical povidone-iodine. *Ophthalmol-*

ogy 98 : 1769-1775, 1991
27) Mistlberger A, Ruckhofer J, Raithel E, Muller M, Alzner E, Egger SF, Grabner G : Anterior chamber contamination during cataract surgery with intraocular lens implantation. *J Cataract Refract Surg* 23 : 1064-1069, 1997
28) Thomas J, Michelle S, Carol H : Intraocular bacterial contamination during sutureless, small incision, single-port phacoemulsification. *J Cataract Refract Surg* 26 : 1786-1791, 2000
29) Ariyasu RG, Nakamura T, Trousdale MV, Smith RE : Intraoperative bacterial contamination of the aqueous humor. *Ophthalmic Surg* 24 : 367-373, 1993
30) Oguz H, Satici A, Guzey M, Aslan G, Tasci S : Microbiologic analysis of aqueous humor in phacoemulsification. *Jpn J Ophthalmol* 43 : 162-165, 1999
31) Hara T, Hoshi N, Hara T : Changes in bacterial strains before and after cataract surgery. *Ophthalmology* 103 : 1876-1879, 1996
32) Sunaric-Mégevand G, Pournaras CJ : Current approach to postoperative endophthalmitis [review]. *Br J Ophthalmol* 81 : 1006-1015, 1997
33) Sherwood DR, Rich WJ, Jacob JS, Hart RJ, Fairchild YL : Bacterial contamination of intraocular and extraocular fluid during extracapsular cataract extraction. *Eye* 3 : 308-312, 1989
34) Dickey JB, Thompson KD, Jay WM : Anterior chamber aspirate cultures after uncomplicated cataract surgery. *Am J Ophthalmol* 112 : 278-282, 1991
35) Egger SF, Huber-Spitzy V, Skorpik C, Weghaupt H, Scholda C, Arocker-Mettinger E, Schneider B, Grabner G : Different techniques of extracapsular cataract extraction : Bacterial contamination during surgery. Prospective study on 230 consecutive patients. *Graefes Arch Clin Exp Ophthalmol* 232 : 308-311, 1994
36) Timo T, Päivi L, Tiina K, Päivi P, Tervo T, Ljungberg P, Kautiainen T, Puska P, Lehto I, Raivio I, Jarvinen E, Kuusela P, Tarkkanen A : Prospective evaluation of external ocular microbial growth and aqueous humor contamination during cataract surgery. *J Cataract Refract Surg* 25 : 65-71, 1999
37) Hatano H, Sasaki T, Tanaka N : Pseudomonas endophthalmitis in rabbits. Intravitreal inoculation of two pseudomonas strains. *Nippon Ganka Gakkai Zasshi* 92 : 1758-1764, 1988 (J)
38) Beyer TL, Vogler G, Sharma D, O'Donnell FE : Protective barrier effect on the posterior lens capsule in exogenous bacterial endophthalmitis. An experimental primate study. *Invest Ophthalmol Vis Sci* 25 : 108-116, 1994
39) Beyer TL, O'Donnell FE, Goncalves V, Singh R : Role of posterior capsule in the prevention of postoperative bacterial endophthalmitis. Experimental primate studies and clinical implications. *Br J Ophthalmol* 69 : 841-846, 1985
40) Hatano H : Experimental Pseudomonas endophthalmitis in rabbits. Intracameral inoculation of two pseudomonas strains. *Nippon Ganka Gakkai Zasshi* 86 : 839-845, 1982 (J)
41) Speaker MG, Milch FA, Shah MK, Eisner W, Kreiswirth BN : Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. *Ophthalmology* 98 : 639-649, 1991
42) Heaven CJ, Mann PJ, Boase DL : Endophthalmitis following extracapsular cataract surgery: A review of 32 cases. *Br J Ophthalmol* 76 : 419-423, 1992
43) Outbreaks of postoperative bacterial endophthalmitis caused by intrinsically contaminated ophthalmic solutions-Thailand, 1992, and Canada, 1993. *MMWR Morb Mortal Wkly Rep* 45 : 491-494, 1996
44) Hoffer KJ : Effect of extracapsular implant techniques on endothelial density. *Arch Ophthalmol* 100 : 791-792, 1982
45) Tanaka T, Inoue H, Kudo S, Ogawa T : Relationship between postoperative intraocular pressure elevation and residual sodium hyaluronate following phacoemulsification and aspiration. *J Cataract Refract Surg* 23 : 284-288, 1997
46) Phillips WB II, Tasman WS : Postoperative endophthalmitis in association with diabetes mellitus. *Ophthalmology* 101 : 508-518, 1994
47) Pleyer U, Mondino BJ, Adamu SA, Pitchekian-Halabi H, Engstrom RE, Glasgow BJ : Immune response to *Staphylococcus* epidermidis-induced endophthalmitis in a rabbit model. *Invest Ophthalmol Vis Sci* 33 : 2650-2663, 1992
48) Quie PG, Belani KK : Coagulase-negative staphylococcal adherence and persistence. *J Infect Dis* 156 : 543-547, 1987
49) Scott IU, Flynn HW Jr, Feuer W : Endophthalmitis after secondary intraocular lens implantation : A case-control study. *Ophthalmology* 102 : 1925-1931, 1995
50) Trivedi RH, Werner L, Apple DJ, Izak AM, Pandey SK, Macky TA : Viscoanesthesia. Part I : Toxicity to corneal endothelial cells in a rabbit model. *J Cataract Refract Surg* 29 : 550-555, 2003
51) Macky TA, Werner L, Apple DJ, Izak AM, Pandey SK, Trivedi RH : Viscoanesthesia. Part II : Toxicity to intraocular structures after phacoemulsification in a rabbit model. *J Cataract Refract Surg* 29 : 556-562, 2003
52) Pandey SK, Werner L, Apple DJ, Izak AM, Trivedi RH, Macky TA : Viscoanesthesia. Part III : Removal time of OVD/viscoanesthetic solutions from the capsular bag of postmortem human eyes. *J Cataract Refract Surg* 29 : 563-567, 2003
53) Moreira CA Jr, Armstrong DK, Jelliffe RW, Moreira AT, Woodford CC, Liggett PE, Trousdale MD : Sodium hyaluronate as a carrier for intravitreal gentamicin. An experimental study. *Acta Ophthalmol* (Copenh) 69 : 45-49, 1991
54) Moreira CA Jr, Moreira AT, Armstrong DK,

Jelliffe RW, Woodford CC, Liggett PE, Trousdale MD: *In vitro* and *in vivo* studies with sodium hyaluronate as a carrier for intraocular gentamicin. *Acta Ophthalmol* (Copenh) 69 : 50-56, 1991

(J): in Japanese